CHAPTER 5
DISCUSSION

Chapter 5: Discussion

Discussion and future perspectives

Anna Posafalvi

Cardiomyopathy is an insidious disease of the myocardium, which can manifest with a wide range of symptoms at various ages, but which usually presents in adulthood. Currently, there are 76 genes known to be involved in the familial form of this disease (for an overview of known disease genes, see the preface of this thesis). Cardiomyopathy has several subtypes in which impairment of various molecular pathways leads to insufficient circulation (as reviewed by Teekakirikul et al). Hypertrophic cardiomyopathy (HCM) was initially thought to be primarily a disease of sarcomeric proteins, while arrhythmogenic right ventricular cardiomyopathy (ARCV) was considered mostly a disease of the desmosomal complex. Restrictive cardiomyopathy has been frequently shown to be caused by desmin (and sometimes sarcomeric) mutations. In addition to these molecules, a large number of proteins responsible for the construction of the cytoskeleton and the nuclear envelope or having a role in calcium/sodium handling have been shown to be involved in dilated cardiomyopathy (DCM) (see review by Posafalvi et al). However, there is increasing evidence that it is not only the phenotypic characteristics of these cardiomyopathy subtypes that are entwined and overlapping, but that the same overlapping pattern is present in their genetic background, as mutations of known genes are increasingly discovered to underlie other subtypes of the disease (Teekakirikul et al). An example of this overlap is described in chapter 4.1 of this thesis: our diagnostic screening of 55 genes implicated in different types of cardiomyopathy led to the discovery of potentially pathogenic variants in genes that would not have been chosen for sequencing in the earlier Sanger-sequencing era. At that time, decisions about which genes to sequence were made based on the clinical phenotype of the patients, and screening was limited to a small number of genes per patient. In addition to the examples of genes now shown to be involved in previously unexpected cardiomyopathy subtypes (reported in chapter 4.1), the complicated genetic overlap among the types of disorder that were already known is visualized in figure 2 of the preface.

Traditionally, Sanger-sequencing of a few disease genes was the standard method used in genetic diagnostics of cardiomyopathies, and the same method was also applied to the screening of novel candidate genes in a research setting. The recent development of whole genome, exome, and gene panel-based high-throughput sequencing technologies created revolutionary possibilities for both diagnostic and research-related applications. The work described in this thesis shows the recent impact of these technical developments on diagnostics and research in cardiogenetics.

Due to the difficulty of defining a small and distinct set of candidate genes to be screened based purely on the specific phenotypic features that a familial cardiomyopathy patient exhibits, we currently apply the gene panel-based targeted-next-generation sequencing described in chapter 4 in the routine DNA diagnostics of cardiomyopathies. Using this technique we are able to sequence 55 well-established disease genes in one experiment, and are in some cases able to identify the genetic explanation of the disease in a gene which would not have been chosen for screening by classical Sanger-sequencing based on the patient's phenotype (for examples, see chapter 4.1). In those individuals whose cardiac health problems could not be explained by genetic variation in the 55 known genes, exome sequencing combined with a haplotype sharing test (when appropriate and depending on the size of the family) seemed to be an effective way of searching for novel candidate disease genes (as shown in chapter 3) that can later be searched for in screens of larger cardiomyopathy cohorts by Sanger-sequencing (as shown in chapter 2). This approach requires phenotypically well-characterized, multi-generational families in which the affected/healthy disease status of individuals has been clearly determined. A flowchart of the cardiogenetics workflow as currently applied in our department is shown in figure 1.

How can we know that we have found the true causative variant?

When sequencing a set of 55 disease genes, there is a fair chance that we will identify likely causal genetic variants in at least one of them. For this reason, we need to take further steps to verify that the variant we are looking at is truly the cause of the patient's disease. After checking the *predicted pathogenicity* of the variant using multiple software packages, the presence/absence and (if applicable) *frequency* of the variant in different population frequency databases (such as GoNL, and the slightly more critically handled dbSNP, 1000G or ESP, which may contain causative variants as well) and performing *segregation analysis* in the family (checking if all affected family members carry the putative causative variant), we might be able to finally classify the variant as 'benign', 'likely benign', 'variant of unknown significance', 'likely pathogenic' or 'pathogenic'. In this thesis, we have used strict and robust criteria for *variant classification* (see chapters 2 and 4 for description and examples). Additionally, we may easily screen a *patient cohort* to search for an additional carrier of the same variant, and if we identify further (unrelated) patients carrying the same mutation, we would apply *haplotype analysis* in the hope of discovering a potential founder effect.

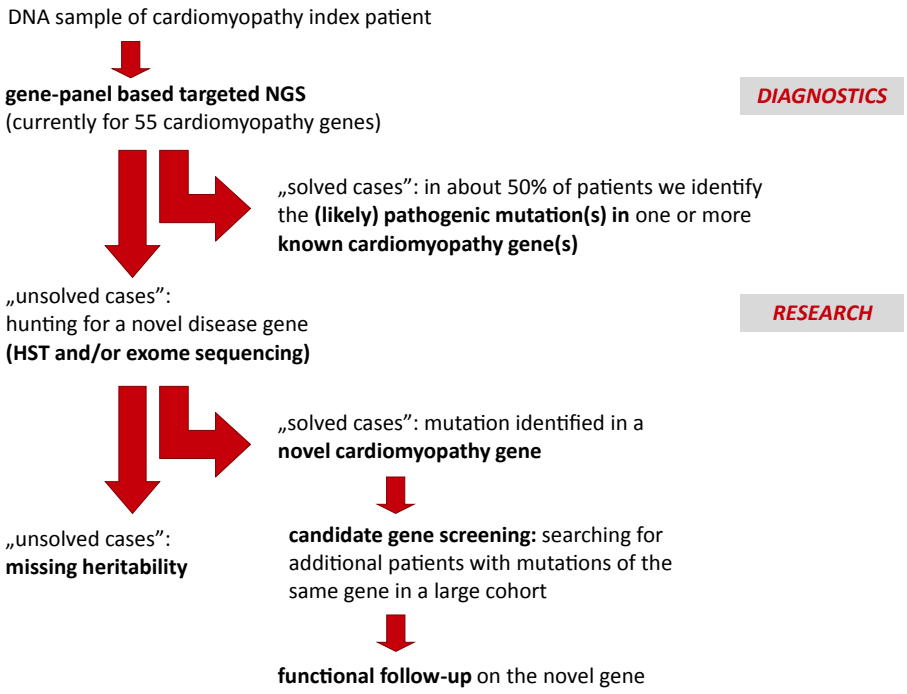

Figure 1: Current cardiogenetics workflow
Since gene panel-based targeted-sequencing is a straightforward approach that sequences all cardiomyopathy disease genes in one experiment, we now implement this as a routine diagnostic screening test. Unsolved cases might later be subject to haplotype sharing analysis, whole exome or genome sequencing, or other disease-gene hunting methods. Novel disease genes identified in these ways are then Sanger-sequenced in large patient cohorts (although we might expect some of these to be private mutations/genes in the families examined), and may be further investigated functionally. In order to ensure up-to-date DNA diagnostics, newly discovered and well-established disease genes can periodically be added to the targeted enrichment kit used for gene panel-based sequencing.

In order to have a more precise idea of the potential pathogenicity level of genetic variants, and to be able to better prioritize variants in large datasets (e.g. as a result of exome/genome sequencing), it is crucial that more reliable and standardized *prediction programs* and software become available. For example, the novel Combined Annotation-Dependent Depletion tool seems to outperform existing software and sources in predicting deleteriousness via incorporating known databases and tools as well as results of the ENCODE project (Kircher et al). Other bioinformatics tools such as well-established *annotation databases* (a good example is the Cardiovascular Gene Ontology Annotation Initiative) and *network tools* (for instance the *co-expression*

network Cytoscape, or protein interaction networks which contain *functional information* on the genes supported by the literature) have also proven to be of great utility. Chapter 3.1 is a straightforward demonstration of how to use such sources in interpreting high-throughput sequencing data. Ultimately, the best way to prove pathogenicity is to perform *functional studies* on the identified variants themselves, and examples of functional analyses via *in vitro* experiments are also described in this thesis. In chapter 2.1 we show how we tried to experimentally evaluate the expected pathogenicity of *RBM20* variants and mutations using a splicing assay. In chapter 3.2 we measured the enzyme activity of superoxide dismutase in patient-derived fibroblasts in order to prove the pathogenicity of a missense variant of the Mn-binding pocket. Finally, in chapter 4.2 we analysed the titin isoform composition and passive force generation of single cardiomyocytes isolated from explanted tissue of a *TTN* frameshift mutation carrier.

There are other ways of acquiring further evidence on the pathogenic nature of the detected genetic variant via functional analysis. A popular but time-intensive method is to set up a knock out/knock in gene in an animal model. Examples for the use of such models to gain more knowledge about the general function of a gene or protein related to the content of this thesis are

- the *RBM20* knock out rat, which has been used for the identification of target RNA molecules of the spliceosomal *RBM20* via sequencing of RNA isolated from heart biopsies of mutant and wild type animals (Guo et al)
- the null-mutant, tissue- and isoform-specific knock out *PLEC* mice, from which much has been learned about the function of plectin in the past decades (Winter et al)
- the lethal mice and *Drosophila SOD2* knock outs suggesting the essential role of this enzyme in the heart (Li et al, Kirby et al)
- the zebrafish model showing the effect of *COBL* knock out on embryonal development of the neural tube and heart (Ravanelli et al).

However, creating an animal model carrying the homolog of the investigated gene with an identical mutation to the one our patient carries is usually a complicated job, which only the recent development of novel gene targeting technologies (TALEN and CRISPR/cas) makes more feasible (Menke 2013). Alternatively, fibroblasts may be acquired from the patient (via a 'simple' skin biopsy), reprogrammed through iPS cells then differentiated into specific cell types such as cardiomyocytes. These cells will be genetically (and also theoretically phenotypically) identical to those in the heart of the

patient, yet are acquired in a less invasive way than a cardiac biopsy sample obtained via catheterization. The derived cells can be used to examine arrhythmogenic cardiac phenotypes and the underlying molecular pathways, as well as to investigate potential opportunities for personalized and/or regenerative therapy. However, this novel technique has been criticized for the low yield of cardiomyocytes produced, their tendency to dedifferentiate and the immature electrophysiological character of the derived cells. It is of crucial importance that the derived cardiomyocytes contain plenty of the genetic variants (mutations and polymorphisms alike) carried by the patient. Therefore, the combination of this technique with a rescue experiment is necessary to exclude other variants from the disease pathomechanism and to prove the sole pathogenicity of the candidate variant under investigation (Sinnecker et al, Knollmann et al).

Where is the "missing heritability" and what indications do we have of the mechanisms in cardiomyopathies?

To date, a significant proportion of familial cardiomyopathies (about 30-40% of HCM, 40-50% of ARVC, and around 50% of DCM cases) remain genetically unexplained. Below we describe some of the possible underappreciated mechanisms that might be behind the "missing heritability" for cardio-myopathies on the DNA, RNA and protein levels, respectively.

1. On the DNA level

In past decades, cardiomyopathy was primarily considered a monogenic disorder, most often exhibiting an autosomal dominant pattern. In the families that do not carry mutations of the 76 disease genes identified so far, we can search for novel candidate genes by exome or whole genome sequencing, when appropriate, in combination with the haplotype sharing test. It is important to keep in mind that some of these families might carry private mutations and no additional affected carriers will be identified in follow-up screening of large patient cohorts. Chapter 3 shows some examples of how we tried to identify novel disease genes in one autosomal recessive family and several autosomal dominant families, with the latter group being naturally much more challenging.

The potential **oligogenic background** of late onset heart diseases is an increasingly popular concept, with a growing number of publications in HCM and ARVC supporting this idea. We also discuss it in chapters 2.2 (in which

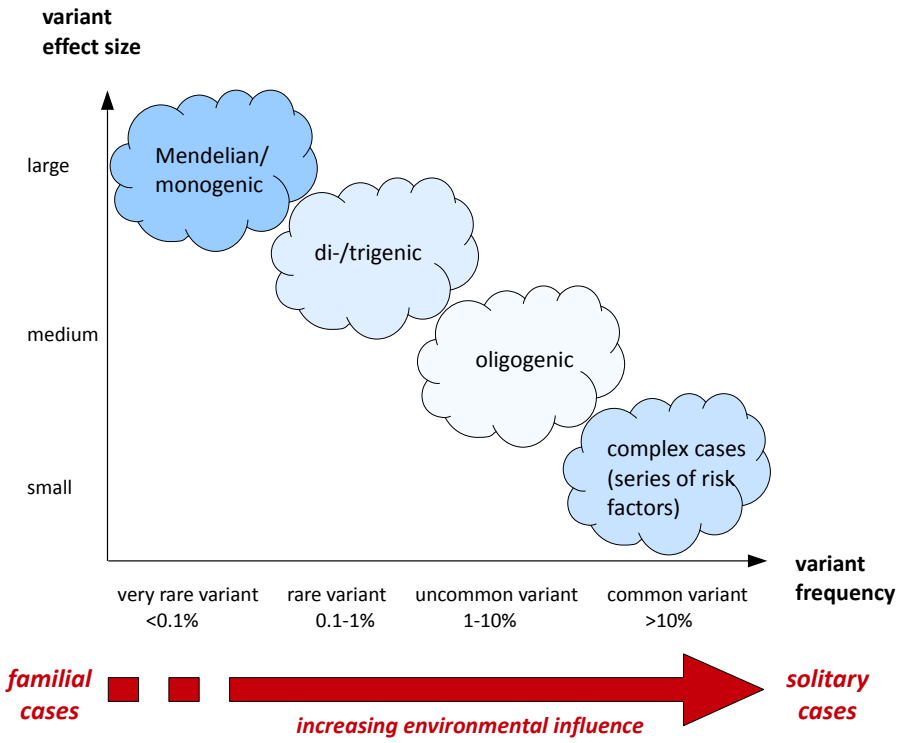

Figure 2: Different disease models in cardiomyopathies
Rare genetic variants of large effect size cause Mendelian, monogenic diseases, while variants of relatively high frequency, but small effect size, are the ones classically identified by GWAS, and associated with certain complex phenotypes. Familial cardiomyopathies are traditionally considered and investigated as monogenic disorders, while a smaller number of studies have tried to establish genetic associations in relatively "large" cohorts of not necessarily familial forms of the disease (between HLA genotypes and cardiac phenotype, for example). Recently there were a few reports of di- and oligogenic cardiomyopathy cases, which suggest the possibility that variants of relatively low frequency and medium effect size may increase an individual's susceptibility to the disease and may also mediate the environmental influence on the disease onset and phenotypic variability.
The degree of darkness of the clouds indicates how well studied those disease models are in cardiomyopathies.

we argue that genetic variants of *PLEC* are expected to be involved in passing the threshold needed for the manifestation of ARVC) and 4.1 (in which we show that 15% of our diagnostically screened patients carry more than one potentially pathogenic variant). Our results suggest that it is not only novel or very rare variants with large effect size that may be implicated in the disease,

but that there are also several low frequency variants with slightly lower effect size that may increase the genetic susceptibility for cardiomyopathy in a non-monogenic disease model (see figure 2). Additionally, the association of cardiomyopathies with complex diseases such as diabetes or coeliac disease has been observed, supporting the idea that alleles of relatively high frequency and low effect size may be involved in a **multifactorial** background of the disease. For example, the increased risk for DCM in patients with coeliac disease (a disease famous of its complex genetics) was apparent, but statistically not significant in a large population-based study (Emilsson et al), while "diabetic cardiomyopathy" is a well-known disease entity that can be treated with targeted antioxidant therapy (reviewed by Huynh et al). There are also a couple of studies that support the idea of the complex genetics of cardiomyopathy by marking the connection between certain *HLA* genotypes (for example, the HLA-DQB1 0309 allele) and DCM (Pankuweit et al). Yet, due to the relatively low incidence of the disease and the low number of affected individuals, it is not easy to perform classical genome-wide association studies (GWAS) in cardiomyopathy while looking for risk or protective factors. It may be possible to compare frequencies of genetic variants of cardiomyopathy genes between patient and control cohorts upon DNA sequencing, but this will require the collection of material from patient cohorts from many different laboratories, while also taking into account the ethnic background of these patients.

The role of **mitochondrial processes** in cardiomyopathies is evident, yet only a few genes related to these processes are shown to be the potential cause of the disease. In this thesis, we have described a mutation of the chromosomally encoded mitochondrial enzyme *SOD2*, which led to lethal cardiomyopathy with additional mitochondrial symptoms in a homozygote newborn (chapter 3.2). There are also mitochondrially encoded tRNA genes that have been reported to be causative, such as the mutation of the gene encoding tRNA glutamic acid that in nearly homoplasmic state proved fatal in an infant (Van Hove et al).

In the case of inherited cardiomyopathies, there has not yet been enough attention paid to possible large **indels** and **copy number variations** (CNVs). There are only a few examples of CNVs identified to date: those of the *BAG3* gene via array CGH (Norton et al), single large deletions of *LMNA* in DCM (Gupta et al) and *PKP2* in ARVC (Li Mura et al), and a large duplication observed in *MYBPC3* in HCM (Meyer T et al). The remaining unsolved affected families may also have duplications, large insertions, or deletions underlying their

phenotypes. Hopefully, in the future, exome and whole genome sequencing methods will provide us with sufficient information on these types of genetic variants.

Epigenetic regulations (the potential mediators of gene-environment interaction via chromatin modification) have never been associated with familial cardiomyopathies. Instead, the influence of the environment is ex-pected to trigger the onset of the phenotype in other ways. For example, pregnancy in individuals who are genetically susceptible for DCM causes earlier onset of the disease (see chapter 4.2), while stress and over-exercising probably contribute to individuals passing the thresholds for the development of an ARVC phenotype (Perrin et al).

2. On the RNA level

The discovery of *RBM20* mutations and the multiple RBM20-target molecules and their heart-specific splicing pattern meant the beginning of a new era in cardiogenetics, and this RNA-based pathway is also closely examined in this thesis (chapter 2.1). Yet we still do not know much about the potential role of **miRNA**s in cardiomyopathy, and the potential **differential splicing effect of variants** of known cardiomyopathy genes is also usually underestimated. These can easily be investigated by RNA sequencing.

Perhaps the most exciting problem related to the role of RNA molecules in cardiomyopathy is that of the titin gene (*TTN).* The longest gene of the human genome, *TTN* has been connected to heart failure (Hein et al) and DCM (Gerull et al) for about two decades, but was never extensively screened due to its enormous size (~0.3Mb). Making things more complicated, *TTN* is not only large, but also has a highly complex pattern of post-translational modifications on the protein level. *TTN* also undergoes random changes on the RNA-level before translation: during its age-dependent splicing it randomly loses a gradually increasing part of the gene between exons 50 and 219 (Guo et al). *TTN* has been recently reported to harbour truncating variants in familial and sporadic DCM (Herman et al), and is nowadays often screened for due to the availability of easy-to-perform gene panel-based sequencing platforms (also shown in chapters 4.1 and 4.2). The inclusion of this gene in DNA-diagnostics resulted in the identification of truncating mutations in ~15% of DCM cases (chapter 4.1). Despite these advances in screening, a problem we continue to face is that we might be underestimating the importance of missense variants. It is possible that the transcribed mRNA

molecules carrying truncating variants are subject to nonsense mediated decay leading to decreased protein production that only becomes a serious issue in homozygous state, while the right missense variant could disrupt a domain or binding site of key importance in the encoded protein and perturb its function in a heterozygous form. Yet despite the existence of some limited literature on functional evaluation of *TTN* missense variants (e.g. a missense mutation of the N2B domain specifically expressed in cardiac isoforms of titin caused a cardiomyopathy-like phenotype in zebrafish (Xu et al)), and further N2B mutations shown to affect the binding of various interacting proteins via yeast-two-hybrid assays by Matsumoto et al), we are biased towards the truncating mutations due to the recent finding of Herman et al that up to 25% of familial DCM is caused by them. In case we identify them localized in one of the exons that might get spliced out in some individuals (hence rescuing the onset of any sort of heart symptoms), it is quite difficult to correctly determine the pathogenicity level even for *TTN* truncations, let alone missense variants. In chapter 4.2, we have performed a functional experiment measuring passive force in single isolated patient cardiomyocytes, and our result supported the "pathogenic" labelling of that studied frameshift variant.

3. On the protein level

Though it has not received much attention thus far, protein aggregation, a current focus in the field of neurodegeneration, may also be related to the pathomechanism of cardiomyopathies. There are examples in the literature showing that certain proteins do form aggregates and are therefore expected to lead to cardiovascular abnormalities. It has, for instance, been previously shown that *PLEC* knock out mouse models as well as skin biopsies of *PLEC* mutant patients with EBS-MD have large, desmin-positive protein aggregates accumulating in their cells (reviewed in Winter & Wiche and also mentioned in chapter 2.2). An exciting, translational potential of this mechanism was demonstrated by the recent discovery that protein aggregation could be inhibited and the phenotype improved in the muscles of plectin deficient conditional knock out mice by the chemical chaperon 4-phenylbutyrate (Winter et al 2014).

Desmin aggregation is also a known phenomenon in heart failure (Sanbe et al) and in desminopathies (myopathies and cardiomyopathies related to abnormal desmin) caused by mutations of *DES* (desmin), *CRYAB* (alpha-B-crystallin or small heat shock protein), *MYOT* (myotilin), *BAG3* (BCL2-

associated athanogene 3), *LDB3* (LIM domain-binding 3), or *FLNC* (filamin C) (reviewed by Goldfarb et al). Interestingly, the expression of the *BAG3* gene (for which a large deletion of about 8 kbp and point mutations have been reported in DCM, Norton et al) was shown to suppress the aggregation and cytotoxic effect of mutant *CRYAB* in cultured cells (Hishiya et al), a discovery that links the two genes to a mutual pathway.

PSEN 1 and *2*, the genes connected to Alzheimer disease as well as cardiomyopathy, are also known to be involved in the formation of amyloid plaques in the myocardium of DCM patients (Gianni et al). Deletions of the *PLN* gene were also recently shown to lead to perinuclear aggregates of the encoded protein in the hearts of deceased DCM and ARVC patients (manuscript submitted). Hopefully, a better understanding of protein aggregation in cardiomyopathies will open up novel possibilities of targeted therapy using various molecules with chaperone activity.

Further aspects and mechanisms

While not yet extensively studied, an interesting observation is that there are some **gender differences** observed in the epidemiology, genetics, and clinical course of autosomal inherited cardiomyopathies (reviewed by Meyer S et al and Fairweather et al). Beyond the environmental influence of the cardiovascular challenges occurring during pregnancy that trigger peripartum cardiomyopathy (PPCM) or DCM at an earlier age in genetically susceptible women (see chapter 4.2), there are also hormone-related pathways involved in the pathomechanism. For example, male *LMNA* carriers are more severely affected than females, and this observation was associated with the nuclear accumulation of androgen receptors in *LMNA* mutant mice (Arimura et al). In contrast, a recent retrospective study found no worsening of symptoms in *LMNA* mutation-carrying women during pregnancy (Palojoki et al). The practical implications of gender differences for the diagnosis, management, and pharmacotherapy of cardiomyopathies were discussed in detail by Fairweather et al.

Another question is how certain genes can be involved in the pathomechanisms of several diseases affecting **multiple organs** leading to a combined phenotype, while in other cases the same genes only cause the disease of one organ. There is a well-known correlation between ARVC, generalized myopathy and various skin diseases. For instance, truncating mutations of *PLEC* cause epidermolysis bullosa, yet missense mutations are observed in

cardiomyopathy without the involvement of blistered skin (chapter 2.2). But there are many other desmosome-related genes also involved in dermatological diseases: for example, mutations in *JUP* cause palmoplantar keratoderma with woolly hair, while in *DSP* they may result in lethal acantholytic epidermolysis bullosa, or skin fragility with woolly hair. Systemic muscular involvement occurs quite frequently in cardiomyopathies: e.g. *LMNA* mutations were found in limb-girdle dystrophy and lipodystrophy besides DCM. *PSEN1* and *PSEN2* genes, when mutated, lead to neurodegeneration (Alzheimer's disease), just as mutations in the potassium channel *KCND3* are associated with another disease of the central nervous system, spinocerebellar ataxia, and with Brugada syndrome (characterized by lethal arrhythmia) (Duarri et al). Mutations in cardiomyopathy genes may affect the health of the sensory organs as well, for instance, in the case of *EYA4* causing hearing loss with DCM and *CRYAB* causing cataract, and/or myofibrillar myopathy with DCM.

...about pharmacogenetics in a nutshell

The basic principle of personalized medicine and pharmacogenetics was created some fifty years ago with the idea that serious side-effects could be prevented and the therapeutic response optimized, if only we were able to give the right medicine in the right dose to the right patient, making the right decision based on his/her individual genetic make-up. Even though genetic research has gone through unprecedented development in the past few decades, and pharmacovigilance databases provide excellent research material for such studies, the number of truly practical implications in patient stratification is still limited.

We have some examples showing the efforts to stratify cardiomyopathy patients, yet these mostly resulted in treatment protocols based not on the genetic background but rather on the symptoms of the patients. For instance, patients suffering from DCM with asymptomatic systolic dysfunction are thought to benefit from pharmacological treatment (Colucci et al). Further examples include the recent observation that PPCM patients may have improved left ventricular ejection fraction when under bromocriptine treatment (Sliwa et al), or that ARVC patients carrying a *PLN* p.Arg14del mutation need the implantation of an ICD earlier than other ARVC patients (van der Zwaag et al).

Based on the genetic background of a patient, classical pharmacodynamic or pharmacokinetic pharmacogenetics could be implemented. Pharmaco-

dynamic pharmacogenetics of cardiomyopathies is not yet a rewarding research field, because cardiomyopathy is usually treated with widely used cardiovascular drugs (such as beta blockers, ACE inhibitors, or calcium channel blockers). These are not known to cause devastating adverse drug reactions (bizarre or type B ADRs) and, if not well tolerated by the patient, are easily replaced by a comparable drug targeting a different pathway. In contrast, pharmacokinetic pharmacogenetics, may be much more promising, because it is of utmost importance that these drugs are administered in the right dose, taking into account the patients' metabolic abilities to achieve optimal blood concentrations of the drug. Genes and the SNPs observed to have an influence on the blood concentration of certain cardiomyopathy medicines could be included in the cardiomyopathy gene panel tests in the future. This would mean that, in parallel with the molecular diagnosis of a cardiomyopathy patient, we could also obtain sequence information to help in immediately adjusting the dose of the drug, and this would facilitate complex counselling (as attempted following personal genome sequencing by Ashley et al). Yet, at this moment, alleles of known SNPs of genes associated with slower/faster drug metabolism can be much faster, cheaper and more easily identified using a genotyping array of limited size. Also, the complete lack of knowledge about the truly functional genetic variants means it is currently not worthwhile to apply sequencing for patient stratification.

In the past decades, another very exciting research area of stratified medicine related to cardiomyopathies has been the struggle to find out why certain drugs used for the treatment of other diseases lead to cardiomyopathy as a result of cardiotoxic side-effects (reviewed by Ky et al). An example is the dilated cardiomyopathy frequently observed after anticancer treatment using anthracycline molecules (briefly touched upon in chapter 3.2). Even though some patients are in danger of being sensitive to the cardiotoxicity of, for example, doxorubicin, they might not have any alternative treatment option available. Different methods of drug formulation, and hopefully better preventive combinations will soon be available to alleviate the toxic side-effects (reviewed by Octavia et al and Carvalho et al).

CONCLUSIONS

Cardiomyopathy is both a clinically and genetically complex disorder. Even though currently 76 genes are known to be involved in the heritable forms of the disease, we cannot explain the familial accumulation of the phenotype

in many cases. This thesis provides an overview of the development of molecular genetic methods implemented during recent years in the research and diagnostics of cardiomyopathies. It contributes to the field through the discovery of novel disease genes as well as through the establishment of new and highly effective methods for molecular diagnostics. In spite of the recent technological advances, the genetic cause of the disease often remains unknown in affected families, as do the complex interactions of environmental and genetic factors. Hopefully the molecular pathways underlying the disease will be extensively studied in the future, ultimately leading to novel translational solutions and practical implications for patients.

REFERENCES

Arimura T, Onoue K, Takahashi-Tanaka Y et al. Nuclear accumulation of androgen receptor in gender difference of dilated cardiomyopathy due to lamin A/C mutations. Cardiovasc Res. 2013;99(3):382-94

Ashley EA, Butte AJ, Wheeler MT et al. Clinical assessment incorporating a personal genome. Lancet 2010;375:1525-35

Carvalho FS, Burgeiro A, Garcia R et al. Doxorubicin-induced cardiotoxicity: from bioenergetic failure and cell death to cardiomyopathy. Med Res Rev 2014;34:106-35

Colucci WS, Kolias TJ, Adams KF et al. Metoprolol reverses left ventricular remodeling in patients with asymptomatic systolic dysfunction: the REversal of VEntricular Remodeling with Toprol-XL (REVERT) trial. Circulation 2007;116(1):49-56

Duarri A, Nibbeling E, Fokkens MR et al. The L450P mutation in KCND3 brings spinocerebellar ataxia and Brugada syndrome closer together. Neurogenetics 2013;14(3-4):257-8

Emilsson L, Andersson B, Elfström P et al. Risk of idiopathic dilated cardiomyopathy in 29 000 patients with celiac disease. J Am Heart Assoc 2012;1(3).001594

Fairweather D, Cooper LT Jr, Blauwet LA. Sex and gender differences in myocarditis and dilated cardiomyopathy. Curr Probl Cardiol 2013;38(1):7-46

Gerull B, Gramlich M, Atherton J et al. Mutations of TTN, encoding the giant muscle filament titin, cause familial dilated cardiomyopathy. Nat Genet 2002;30(2):201-4

Gianni D, Li A, Tesco G et al. Protein aggregates and novel presenilin gene variants in idiopathic dilated cardiomyopathy. Circulation 2010;121(10):1216-26

Goldfarb LG and Dalakas MC. Tragedy in a heartbeat: malfunctioning desmin causes skeletal and cardiac muscle disease. J Clin Invest 2009;119:1806-13

Guo W, Schafer S, Greaser ML et al. RBM20, a gene for hereditary cardiomyopathy, regulates titin splicing. Nat Med 2012;18(5):766-73

Gupta P, Bilinska ZT, Sylvius N et al. Genetic and ultrastructural studies in dilated cardiomyopathy patients: a large deletion in the lamin A/C gene is associated with cardiomyocyte nuclear envelope disruption. Basic Res Cardiol 2010;105:365-377

Hein S, Scholz D, Fujitani N et al. Altered expression of titin and contractile proteins in failing human myocardium. J Mol Cell Cardiol 1994;26(10):1291-306

Herman DS, Lam L, Taylor MR et al. Truncations of titin causing dilated cardiomyopathy. N Engl J Med 2012;366(7):619-628

Hishiya A, Salman MN, Carra S et al. BAG3 directly interacts with mutated alphaB-crystallin to suppress its aggregation and toxicity. PLoS One 2011;6(3):e16828

Huynh K, Bernardo BC, McMullen JR et al. Diabetic cardiomyopathy: Mechanisms and new treatment strategies targeting antioxidant signaling pathways. Pharmacol Ther 2014;142(3):375-415

Kirby K, Hu J, Hilliker AJ et al. RNA interference-mediated silencing of Sod2 in Drosophila leads to early adult-onset mortality and elevated endogenous oxidative stress. Proc Natl Acad Sci USA 2002;99(25):16162-67

Kircher M, Witten DM, Jain P et al. A general framework for estimating the relative patho-

genicity of human genetic variants. Nat Genet 2014;46:310-5

Knollmann BC: Induced pluripotent stem cell-derived cardiomyocytes – Boutique Science or valuable arrhythmia model? Circ Res 2013;112:969-976

Ky B, Vejpongsa P, Yeh ET et al. Emerging paradigms in cardiomyopathies associated with cancer therapies. Circ Res 2013;113:754-64

Li Y, Huang TT, Carlson EJ et al. Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. Nat Genet 1995;11:376-81

Li Mura IE, Bauce B, Nava A et al. Identification of a PKP2 gene deletion in a family with arrhythmogenic right ventricular cardiomyopathy. Eur J Hum Genet 2013;21:1226-31

Limphong P, Zhang H, Christians E et al. Modeling human protein aggregation cardiomyopathy using murine induced pluripotent stem cells. Stem Cells Transl Med 2013;2(3):161-6

Matsumoto Y, Hayashi T, Inagaki N et al. Functional analysis of titin/connectin N2-B mutations found in cardiomyopathy. J Muscle Res Cell Motil 2005;26:367-74

Menke DB: Engineering subtle targeted mutations into the mouse genome. Genesis 2013;51(9):605-18

Meyer S, van der Meer P, van Tintelen JP et al. Sex differences in cardiomyopathies. Eur J Heart Fail. 2014;16(3):238-47

Meyer T, Pankuweit S, Richter A et al. Detection of a large duplication mutation in the mysin-binding protein C3 gene in a case of hypertrophic cardiomyopathy. Gene 2013;527:416-20

Norton N, Li D, Rieder MJ et al. Genome-wide studies of copy number variation and exome sequencing identify rare variants in BAG3 as a cause of dilated cardiomyopathy. Am J Hum Genet 2011;88(3):273-82

Octavia Y, Tocchetti CG, Gabrielson KL et al. Doxorubicin-induced cardiomyopathy: from molecular mechanisms to therapeutic strategies. J Mol Cell Cardiol 2012;52:1213-25

Palojoki E, Kaartinen M, Kaaja R et al. Pregnancy and childbirth in carriers of the lamin A/C-gene mutation. Eur J Heart Fail 2010;12:630-3

Pankuweit S, Ruppert V, Jónsdóttir T et al. The HLA class II allele DQB1 0309 is associated with dilated cardiomyopathy. Gene 2013;531(2):180-3

Perrin MJ, Angaran P, Laksman Z et al. Exercise testing in asymptomatic gene carriers exposes a latent electrical substrate of arrhythmogenic right ventricular cardiomyopathy. J Am Coll Cardiol 2013;62:1772-9

Posafalvi A, Herkert JC, Sinke RJ et al. Clinical Utility gene card for: dilated cardiomyopathy (CMD). Eur J Hum Genet 2012; doi:10.1038/ejhg.2012.276

Ravanelli AM & Klingensmith J: The actin nucleator Cordon-bleu is required for development of motile cilia in zebrafish. Dev Biol 2011;350(1):101-11

Sanbe A, Osinska H, Saffitz JE et al. Desmin-related cardiomyopathy in transgenic mice: a cardiac amyloidosis. Proc Natl Acad Sci USA 2004;101:10132-6

Sliwa K, Blauwet L, Tibazarwa K et al. Evaluation of bromocriptine in the treatment of acute severe peripartum cardiomyopathy: a proof-of-concept pilot study. Circulation 2010;121(13):1465-73

Sinnecker D, Goedel A, Laugwitz KL et al. Induced pluripotent stem cell-derived cardiomyocytes – A versatile tool for arrhythmia research. Circ Res 2013;112:961-968

Teekakirikul P, Kelly MA, Rehm HL et al. Inherited cardiomyopathies: molecular genetics and clinical genetic testing in the postgenomic era. J Mol Diagn 2013;15(2):158-170

van der Zwaag PA, van Rijsingen IA, de Ruiter R et al. Recurrent and founder mutations in the Netherlands-Phospholamban p.Arg14del mutation causes arrhythmogenic cardiomyopathy. Neth Heart J 2013;21(6):286-93

Van Hove JL, Freehauf C, Miyamoto S et al. Infantile cardiomyopathy caused by the T14709C mutation in the mitochondrial tRNA glutamic acid gene. Eur J Pediatr 2008;167(7):771-6

Winter L & Wiche G: The many faces of plectin and plectinopathies: pathology and mechanisms. Acta Neuropathol 2013;125(1):77-93

Winter L, Staszewska I, Mihailovska E et al. Chemical chaperone ameliorates pathological protein aggregation in plectin-deficient muscle. J Clin Invest 2014;124(3):1144-57)

Xu X, Meiler SE, Zhong TP et al. Cardiomyopathy in zebrafish due to mutation in an alternatively spliced exon of titin. Nat Genet 2002;30:205-9